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Experimental Lumbar Spine Fusion With Novel Tantalum-Coated Carbon Fiber Implant

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Abstract: Implants of carbon fiber composite have been widely used in orthopedic and spinal surgeries. However, studies using carbon fiber-reinforced cages demonstrate frequent appearance of fibrous layer interposed between the implant and the surrounding bone. The aim of the present study was to test the possibility of coating a biocompatible metal layer on top of the carbon fiber material, to improve its biological performance. Tantalum was chosen because of its bone compatibility, based on our previous studies. A novel spinal fusion cage was fabricated by applying a thin tantalum coating on the surface of carbon–carbon composite material through chemical vapor deposition. Mechanical and biological performance was tested *in vitro* and *in vivo*. Compress strength was found to be 4.9 kN (SD, 0.2). Fatigue test with 500,000 cycles was passed. *In vitro* radiological evaluation demonstrated good compatibility with X-ray and CT scan examinations. *In vivo* test employed eight pigs weighing 50 kg each. Instrumented lumbar spine fusion of L3/4 and L4/5 with these cages was performed on each pig. After 3 months, excellent bone integration property was demonstrated by direct contact of the cage with the host bone and newly formed bone. No inflammatory cells were found around the implant. Cages packed with two different graft materials (autograft and COLLOSS) achieved the same new bone formation. The present study proved that coating tantalum on top of the carbon-based implant is feasible, and good bone integration could be achieved. J Biomed Mater Res Part B: Appl Biomater 81B: 194–200, 2007

Keywords: tantalum; carbon fiber; spine fusion; implant; interface

INTRODUCTION

Subsequent to Bagby's pioneering work in the introduction of cages in spinal interbody fusion in 1988,[1] many new cages have been, and continue to be developed. Regardless of their various designs, the main aim of cages is to fuse the two adjacent vertebrae together, thus eliminating symptoms by providing stability to the spinal segment. Cage design has been focused on geometry, shape, initial stabilities, and mechanical properties.[2] However, the bone–cage interface, in terms of bone integration, has not been adequately addressed.

Carbon fiber-reinforced spinal fusion cages (CFRC) have been used widely in clinical practice, with the advantages of radio-transparency and elasticity similar to that of bone.[3,4] From our own experience, the bone–cage interface of CFRC has been inconsistent and unsatisfactory.[5,6] Hojo et al.[7] also reported that CFRC was often encircled by a thick fibrous tissue layer.

A way to circumvent this bone integration problem is to apply a biocompatible metal layer on the surface of the carbon fiber cage. The excellent bone integration results of porous tantalum cage from our previous studies[6,8] made us believe that a thin layer of tantalum coating could improve the bone–cage interface while preserving the good mechanical and radiological properties. Tantalum has been in clinical use since 1940, and has found a wide range of diagnostic and implant applications, with apparently overall excellent results.[9] Tantalum can be applied as a coating by means of various techniques, including chemical vapor deposition (CVD), molten salt electrodeposition, or physical vapor deposition.

In the present study, we tested the performance of tantalum-coated carbon fiber cage in the porcine lumbar spinal fusion model, and, in the meantime, a bovine bone collagen extract was tested as a bone graft substitute inside the cage.

MATERIALS AND METHODS

Carbon Fiber Cage With Tantalum Coating

The carbon fiber-reinforced carbon composite was AC 150 (Across, Japan). The experimental spinal cages were machined

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Figure 1. Cage design and shape.

to a shape similar to that of the Brantigan I/F cage (DePuy Acromed, Raynham, MA) by Carbon Industrie-produkte GmbH, Germany, with dimensions of 9 mm (posterior height) × 20 mm (width) (Figure 1). Tantalum coating was provided by Danfoss Tantalum Technologies in Denmark. The tantalum coating was applied by means of the CVD[10] process. The coating thickness was optimized and validated by radiographic evaluation. Micro CT and MRI were also employed to evaluate the prototype of the implant.

Mechanical Test and *In Vitro* Imaging Assessment

Mechanical tests of compressive strength, compressive fatigue strength, and simulation implantation were performed. The compressive strength was determined by mounting the cage to the test machine (Instron, 6025, USA) with tapered polyethylene blocks, to mimic a flexible spine. An axial force at a rate of 500 N/min was used for testing. The loading was stopped either when a permanent failure of the specimen occurred or when a displacement of 3.0 mm was reached. Compressive fatigue strength was verified using the same test set-up, with a cyclic loading between 400 and 2000 N for 500,000 cycles. Simulated implantation was performed by manipulating the cages under a compression force of 400 N. Imaging assessments were carried out on prototype, by subjecting the implant to radiograph, micro-CT, and MR examinations.

Animals and Study Design

Eight normal Danish landrace pigs with an average weight of 50 kg were used in this experiment. Lumbar spine interbody fusion of L3/4 and L4/5, using tantalum-coated carbon–carbon composite (TCC) cages and pedicle screw fixation, was performed on each pig. The local ethical committee for animal experiments under the J.nr.1998-561-67 has approved the study protocol. A bovine bone protein extract (COLLOSS®, OSSACUR AG, Oberstenfeld, Germany) was tested inside the cage as a bone graft substitute. Cages packed with either autograft or COLLOSS were randomly assigned to the two fusion levels. Pigs were followed for 3 months before termination.

Anesthesia and Surgery

Anesthesia and surgical procedures are described in detail in our previous publications. Briefly, under general anaesthesia, autologous bone graft was taken from the iliac crest, with the pig placed in a prone position. With the same position, posterior pedicle screw fixation was also engaged by taking an intermuscular approach. Pedicle screw instrumentation (Ti6Al4V, 3.5*5, Meditronic, Sofamor Danek, Minneapolis, MN) was performed between L3 and L5 on each pig under a C-arm fluoroscopy. The pig was then moved to a supine position, and a left paramedian 15–20 cm long abdominal incision was made. Via a retroperitoneal approach, the anterior lumbar spine was exposed. Following ligation of the segmental vessels, intervertebral discs of L3/4 and L4/5 together with vertebral physeal plates were removed. Two tantalum-coated C—C cages, packed with either autograft or COLLOSS, were inserted into the prepared disc space according to a predesigned random table. After a careful check of the abdominal cavity, the abdominal wall was carefully sutured by layers. Pigs were housed separately with *ad libitum* access to water. After 3 months observation, they were killed under general anesthesia by means of intravenously administered pentobarbital. Spine segments from L1 to sacrum were taken, stripped of soft tissue, and frozen at −20°C until examination.

Radiograph and Micro-CT Evaluation

Radiographs of double projections were followed at 4, 8, and 12 weeks postoperatively. After termination, all lumbar specimens were subjected to clinical CT scanning (1.5T, MX8000, Marconi, USA) with 2-mm thick slices and 1-mm increments. Specimens of cages, together with the neighboring vertebral bone, were then prepared by means of precision sawing. The bone–cage blocks were scanned by high-resolution micro-CT scanning (μ-CT 40, Scanco Medical AG., Zürich, Switzerland). The scanned images had a three-dimensional (3-D) reconstruction of cubic voxel sizes, $38 \times 38 \times 38\ \mu m^3$. Each 3-D image dataset consisted of ~200 micro-CT slide images (1024 × 1024 pixels) with 16-bit-gray-levels. Fusion was defined as continuous trabeculae bridging across the cage space. From accurate 3-D datasets, bone volume fraction (BV/TV) and trabecular thickness (TbTh) were calculated based on unbiased, assumption-free 3-D methods.

Histomorphometry

Following the micro CT scanning, the bone–cage blocks were dehydrated in graded ethanol (70–99%) containing

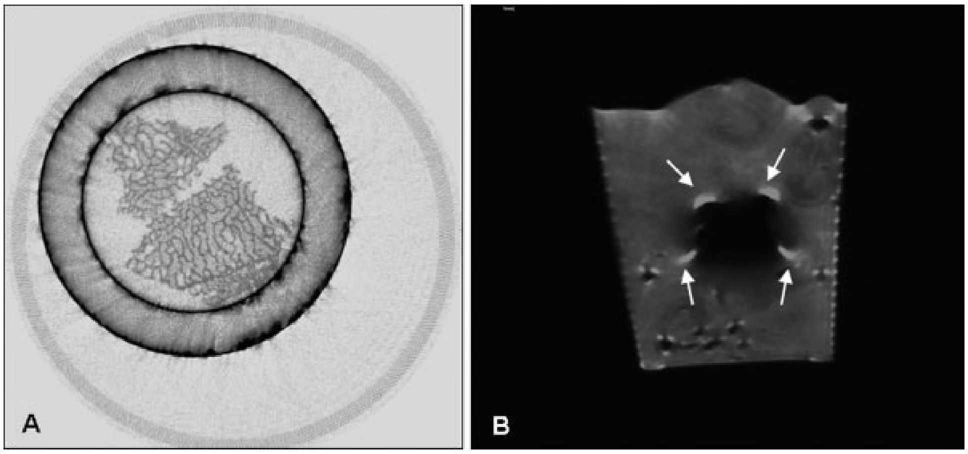

Figure 2. (A) Micro-CT image of the prototype implant with bone chips inside. Trabeculae structure can be clearly visualized. (B) MR scanning of the implant that submerged in water. T1-weighted images showed scattering artifact around the implant edge (arrows).

0.4% basic Fuchsin, and embedded in PMMA. They were cut to a thickness of 40–50 μm using the sawing microtome KDG 95 (Meprotech, Heerhugowaard, Netherlands). The surface was counterstained with 2% light green for 2 min. Four coronal sections were produced from each bone–cage sample, with 500-μm steps. Histological sections were read under the light microscope to define new bone, cartilage, and fibrous tissue. Blinded quantitative evaluation was performed using the points count technique by capturing the histological images with a 3-CCD video camera to the computer (CAST-grid system, Olympus Denmark A/S, Glostrup, Denmark). New bone volume, bone marrow, cartilage tissue and fibrous tissue volumes were calculated in percentage of the specific volume inside the cage.

Statistics

Data were analyzed by means of SPSS and presented as mean $\pm$ SD. A normality test (Q-Q plot) for approximation to normal distribution was used. Based on the self-controlled study design, micro-CT and histomorphometrical results were compared by paired *t*-test. $p < 0.05$ (two-tailed) was considered significant.

RESULTS

Mechanical Properties

The compressive strength of the TCC cages was determined to be 4,9 $\pm$ 0.2 kN (eight samples). All the tested samples passed the compressive fatigue test, 400–2000 N cyclic load for 5 million cycles. In the *in vivo* test, all the pigs survived the operation and observation. One pig was excluded at 8 weeks' checkup because of implant-related complications.

Radiological Assessment

The coating thickness could greatly affect the radio-transparency. The final coating thickness of 0.5 $\pm$ 0.3 μm was

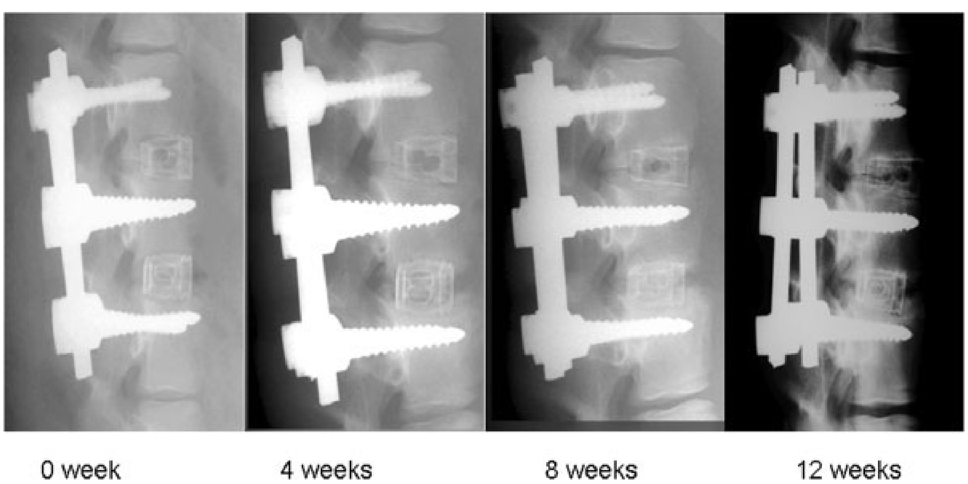

Figure 3. Serial X-ray examinations from immediate postoperation to 12 weeks after. Images show that the lower level (L4/5) has a slightly higher density.

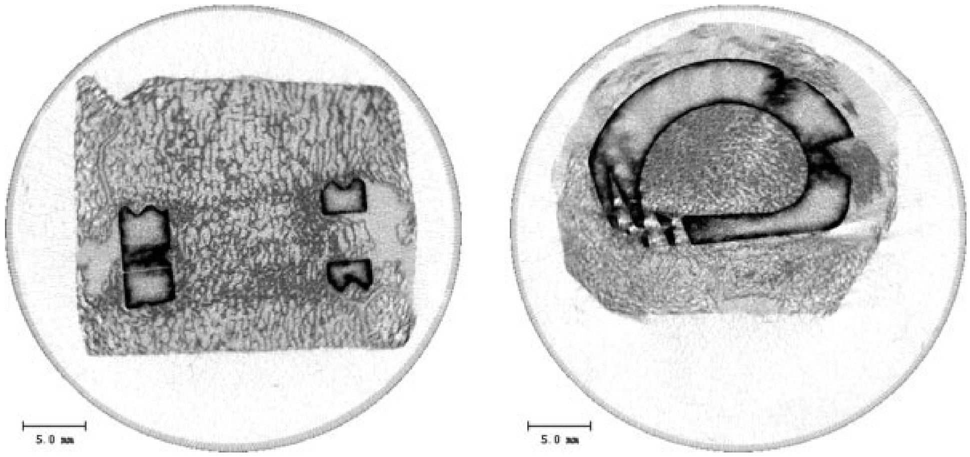

Figure 4. Micro CT images of both sagittal (left) and axial (right) scanning demonstrate excellent bone-implant contact.

chosen, to obtain optimum image quality. This thin coating did not demonstrate notable artifact on micro CT evaluation [Figure 2(A)], while small artifacts were found on the edge of the implant with MR scanning *in vitro* [Figure 2(B)]. *In vivo*, all the cages demonstrated good radio-transparency for serial evaluation of bone formation inside (Figure 3). Clinical CT evaluation of fusion showed that fusion rate for COLLOSS-packed cages was 57% (4/7) and for the autograft was 100% (7/7). Excellent biocompatibility was demonstrated by micro-CT images, in which bone in direct contact with the Ta-coated cages was abundant (Figure 4). With reconstructed micro-CT images, fusion rate for COLLOSS packed cages improved to 85.7% (6/7). Micro-CT evaluation showed that there were no differences in the BV/TV, surface densities (BS/BV), and trabecular thickness (TbTh) between the two graft materials. Only trabecular space (TbSp) and trabecular number (TbN) had significant differences between them ($p = 0.02$ and $p = 0.03$, respectively) (Table I).

Histology and Histomorphometry

On macro-examination of the spine samples, there was no sign of inflammation or discoloration around the implants. Histology sections demonstrate intimate contact of trabecular bone to the cage surface (Figure 5). There were no signs of inflammatory cell infiltration or giant cells around the implant. Bone structure formed inside the cage was similar to that outside the cage, with only slight condensation near the implant. Quantitative analysis with histomorphometry showed that the autograft-packed cages had a higher amount of bone marrow space ($p = 0.047$) and lower amount of cartilage tissue volume ($p = 0.002$). Differences between bone volume and fibrous tissue volume were not significant (Figure 6).

TABLE I. Micro CT Evaluation Results of Both Autograft Bone and COLLOSS Filled Cages

	Autograft	Colloss	p Value
BS (mm)	3765.37 (436.18)[a]	2779.50 (1188.00)	0.14
BV (mm^3)	423.29 (116.33)	285.46 (119.30)	0.14
TV (mm^3)	802.08 (65.65)	708.41 (200.82)	0.37
BS/BV (mm^{-1})	9.35 (2.13)	9.83 (2.39)	0.85
BS/TV (mm^{-1})	4.70 (0.43)	3.84 (0.88)	0.07
BV/TV (%)	0.525 (0.12)	0.41 (0.12)	0.15
TbTh (μm)	0.24 (0.04)	0.23 (0.04)	0.86
TbSp (μm)	0.34 (0.07)	0.76 (0.38)	0.03*
TbN (mm^{-1})	3.05 (0.48)	1.86 (0.70)	0.02*

* Paired t-test.
[a] Values in parentheses indicate SDs.

DISCUSSION

The concept of coating a metal layer on top of carbon fiber-reinforced implant proved to be feasible, and the biological results are promising in the present experiment. The TCC cages demonstrated adequate mechanical properties to sustain the load, in addition to excellent biocompatibility for bone integration. Different graft materials, autograft and COLLOSS, achieved the same new bone formation inside the TCC cages.

Considering the mechanical properties, the compressive strength of the TCC cage is comparable to that of a Brantigan cage of similar shape.[11] The fatigue test showed no visible damage to the cage. In simulated implantation test, it passed the insertion and pull out, ±45° twist tests, with holding tool under 400 N preload.

As observed from the radiograph, CT and micro CT images, the radio-transparent property of CFRC was inherited in the present TCC cage. The tantalum coating delineated the cage clearly, facilitating the monitoring of cage position, deformity, or cracking. Both clinical CT scanner and micro CT are applicable in the evaluation of fusion status inside the cage. Furthermore, elasticity of carbon-fiber implant, whose elastic modulus is close to that of cortical bone, was also preserved in the TCC cage. In the present experiment, 13 out of 14 TCC cages achieved fusion after 3 months' observation. The bone quality inside the cage was similar to that outside the cage, in terms of

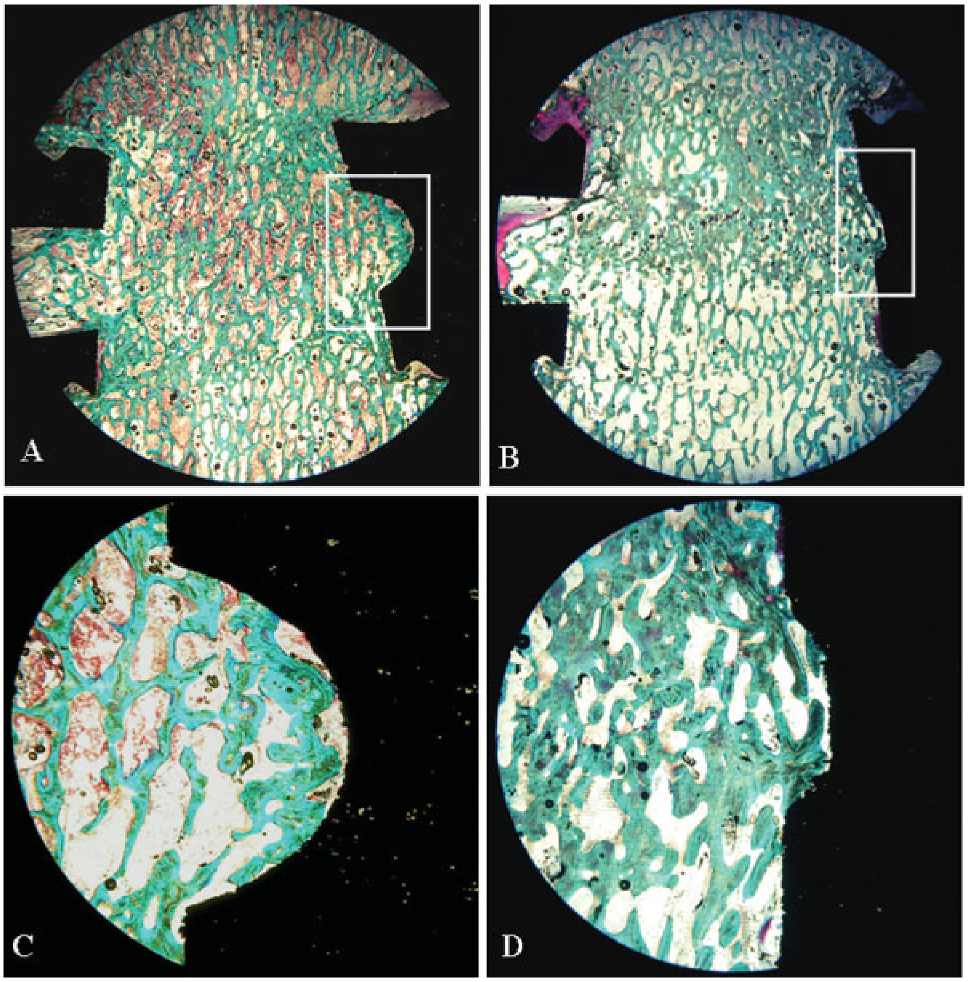

Figure 5. Histological sections showed solid bony fusion in both autograft (A) and COLLOSS (B) filled cages (×6 magnification). Excellent bone integration depicted by direct bone contact is clearly seen when zoomed in at the white frame area (C, D ×20 magnification). Staining: basic fuchsin and light green.

structure and orientation, which was probably due to good elasticity of the implant. COLLOSS achieved the same bone formation, but more cartilage tissue in comparison to that of autograft, which is consistent with our previous results.[12] Given the differences in biomechanics and physiology of spinal fusion between pigs and humans, empty CFRC could not achieve fusion in this pig model after 3 month's observation.[13] This means that the performance of COLLOSS in a TCC cage is promising. However, 3 months' observation time was insufficient to assess the fusion quality or predict the final fusion; if a longer observation time was employed, the cartilage tissue could mineralize and form bone.

Owing to the difference in resolution, fusion was more accurately assessed by micro CT, which was capable of tracing a single trabecula inside the cage. This could explain why two Colloss-packed cages that were diagnosed bone-fusion by clinical CT were actually found to be fused by micro CT. Micro CT is more preferable in terms of evaluating bone formation in the TCC cage, because it scans the whole sample and generates more than 300 images with fine resolution. Furthermore, fresh or freshly frozen specimens were scanned, thus, avoiding the interface damage that could occur with dehydration in routine histological preparation. However, histological sections provided the information of cellular response, cartilage and fibrous tissue formation, which was otherwise difficult to get from micro CT. Histological examination showed no inflammatory or foreign body reaction to the TCC implant, while its biocompatibility was again indicated by large bone contact.

The underlying reason for the effect of tantalum coating is not yet clear. Our previous study demonstrated that surfaces coated with tantalum resulted in an improved metabolic response of mesenchymal stem cells in comparison to glass surface or chromium-coated surface.[14] An ongoing study by the present authors, comparing the metabolic response of osteoblast and mesenchymal stem cells to the tantalum-coated or uncoated carbon fiber surfaces, could provide clues to this effect in future.

One of the main concerns about using carbon fiber implant is that debris is released from the implant. To alleviate free fiber release, the fibers are commonly embedded

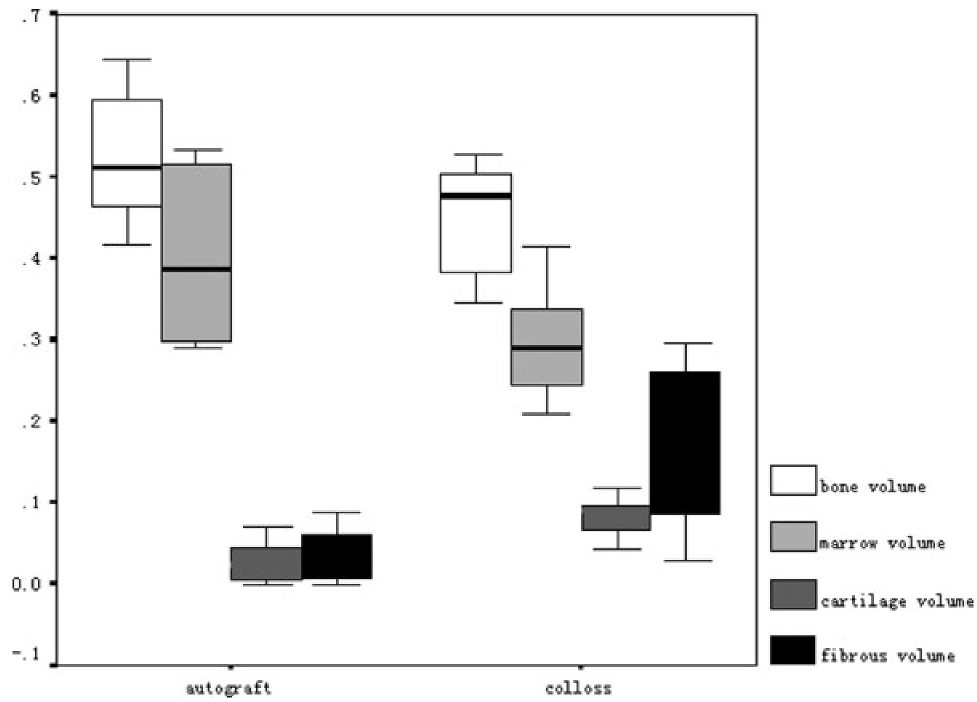

Figure 6. Histomorphometrical comparison of the autograft bone and COLLOSS inside the TCC cages.

in a composite material, such as epoxy resin or polyetheretherketone. Brantigan et al.[15] showed no adverse effect to cage devices in goats, while Belangero et al.[16] found inflammatory infiltration of fibroblasts, macrophages, and giant cells in response to particulate debris in rats. In the present study, no inflammatory reaction was found against the cage structure locally. However, systemic screening of particle release in spleen, kidney, brain, and other internal organs will need a separate study with different time points.

CONCLUSION

Coating a thin layer of tantalum on top of carbon fiber-reinforced implant proved to be feasible. The implant demonstrated sufficient mechanical strength to sustain physiological load. The tantalum coating can serve as a radiological marker and also a surface modification for bone integration.

Danfoss Tantalum Technologies, Lyngby, Denmark provided the cages. OSSACUR AG, Oberstenfeld, Germany provided the COLLOSS.

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